GENETIC RELATEDNESS AMONG CO-FOUNDRESSES OF TWO DESERT ANTS, *VEROMESSOR PERGANDEI* AND *ACROMYRMEX VERSICOLOR* (HYMENOPTERA: FORMICIDAE)

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Cooperative colony foundation occurs in some social hymenoptera. Polistine wasp foundress associations are usually composed of close relatives (reviewed in Gamboa et al. 1986, Michener and Smith 1987) suggesting kin selection may play an important role in establishment of such groups. Cooperative colony foundation, however, may be advantageous even if cofoundresses are not related (Lin and Michener 1972, Pollock and Rissing 1988a). Indeed, several behavioral (reviewed in Rissing and Pollock 1988) and one electrophoretic (Ross and Fletcher 1985) study suggest ant foundress associations form without respect to relatedness. Here we report on an electrophoretic analysis of intra-group relatedness among co-foundresses of *Veromessor pergandei* and *Acromyrmex versicolor,* two common desert ants with cooperative colony foundation (Pollock and Rissing 1985, Rissing and Pollock 1986, Rissing et al. 1986).

Ideally, relatedness should be measured directly through pedigree analysis of interacting individuals (Hamilton 1972). Since this is impractical for most natural populations of social insects, the alternative is indirect estimation using neutral genetic markers (Pamilo and Crozier 1982, Pamilo 1984). We used polymorphic allozyme loci, detected by protein electrophoresis, for this purpose (Richardson et al. 1986). Allozyme loci offer the advantage that homozygous and heterozygous individuals are readily distinguishable; in addition, these loci are not likely involved directly in determining behavior patterns and thus can be treated as selectively neutral within the context of social evolution (Pamilo 1984).

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Materials and Methods

Foundress associations of *V. pergandei* were collected from two sites, "Main" and "Granite" (2 km apart) immediately south of the southeast corner of South Mountain Park, Phoenix, AZ during February–March 1988. Foundress associations of *A. versicolor* were collected from a site in North Scottsdale, AZ (described in Rissing et al. 1986) in September 1987. In each case, existence of a single characteristic mound of freshly excavated soil indicated a single foundress association. Live co-foundresses were air expressed immediately to Michigan State University where they were frozen at −80° C and stored until electrophoresed.

Electrophoretic methods. We prepared frozen ants for electrophoresis by grinding them individually in an extraction buffer at 4° C. We removed the gasters of *V. pergandei* queens before grinding in a pH 7.0, 0.1 M tris buffer (with 40 mg EDTA, 20 mg NAD, 10 mg NADP and 250 μl beta-mercaptoethanol per 100 ml: Buffer 1) or in an unbuffered detergent solution (with 100 μl Triton-X, 10 mg NADP and 100 μl beta-mercaptoethanol per 100 ml: Buffer 2). Buffer 2 gave superior results for esterases but was no better, and in some cases worse, than Buffer 1 for other enzymes. We ground whole *A. versicolor* in buffer 1. For each ant we adjusted the amount of buffer from 10 to 100 μl to give an approximately equal ratio of buffer to ant tissue.

We applied extracts from 12 ants (ca. 1 μl from each) to thin-layer cellulose acetate plates (Titan III: Helena Laboratories, Beaumont, TX). Plates were soaked for at least 30 min in a running buffer before sample application; we used the same buffer for the electrophoretic run. We used cellulose acetate running buffers "A", "B", "C", "D", and "I" of Richardson et. al. (1986); no single buffer gave good resolution for all enzymes tested. Run durations ranged 15–35 minutes, under constant voltage (200–300 V); durations and voltages were adjusted to optimize separation for each enzyme that showed clear activity. Combinations of running buffer, voltage and time giving best results are noted below. All electrophoresis was done at 4° C.

To visualize the allozymes we used enzyme-specific stains (Harris and Hopkinson 1978, Richardson et al. 1986), mixed 1:1 with 1.5% agar solution and poured onto the plates. When sufficient stain intensity was reached, we rinsed off the agar layer and soaked the

plate in tap water to remove unreacted dye. Precipitated dye remains in the cellulose acetate layer, so stained plates were preserved directly or photocopied. Genetic interpretations of variation in resulting bands was based on known enzyme quaternary structure (Harris and Hopkinson 1978, Richardson et al. 1986), supplemented by comparison of haploid males where possible.

We resolved allozyme products of 18 presumptive genetic loci from *V. pergandei* queens, 4 of which were polymorphic with 2 alleles each: *Est-1* and *Est-2* (general esterase; beta-naphthyl acetate as substrate), *Mdh-1* (malate dehydrogenase, EC 1.1.1.37) and *Pgm* (phosphoglucomutase, EC 2.7.5.1). Optimal separation for the *V. pergandei* esterase allozymes was given by buffer I (25 min at 250 V); for *Mdh-1* by buffer C (30 min at 250 V); and for *Pgm* by buffer I (25 min at 250 V). An additional polymorphism for *Idh-1* (isocitrate dehydrogenase, EC 1.1.1.42) was present in one small sample (buffer I, 20 min at 250 V). Banding patterns and allele designations for the esterase loci are shown in Figure 1; esterase genotypes could not be scored from all individuals.

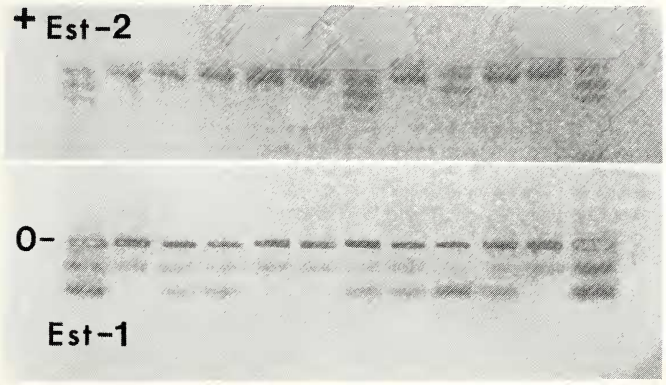

Figure 1. Zymogram of esterase loci in *V. pergandei.* Alleles for *Est-1* are designated "C" (cathodal) and "A" (anodal); the enzyme behaves as a monomer. *Est-1* CC and CA genotypes are distinguished by the relative intensity of each band, since an artifact band comigrates with the A allozyme. *Est-2* has alleles "F" (fast) and "S" (slow) and behaves as a dimer. "O" = origin; "+" = anodal. For *Est-1,* lanes, 2, 5, 6, and 11 are "A/A"; lanes 1, 3, 4, 7, 8 and 10 are "A/C" and lane 9 is "C/C". For *Est-2* lanes 2–6, 7, 10 and 11 are "F/F" and lanes 1, 7, 9 and 12 are "F/S".

An anamalous *Gpi* (glucose phosphate isomerase, EC 5.3.1.9) and *Ao* (aldehyde oxidase, EC 1.2.3.1) banding pattern was present in low frequency among *V. pergandei* queens (buffer I, 25 min at 250 V). Since we were unable to verify Mendelian inheritance of the variant pattern, which could not be interpreted easily in terms of known enzyme quaternary structure, we have omitted these loci from the analysis. The monomorphic loci resolved from *V. pergandei* were (enzyme trivial name, E.C. code and best running buffer are listed after each): *Aat-1* (aspartate aminotransferase, EC 2.6.1.1; I); *Ac* (aconitase, EC 4.2.1.3; C); *Dia* (diaphorase, EC 1.6.*.*; D); *G3p* (glycerol-3-phosphate dehydrogenase, EC 1.1.1.8; A); *G6pdh* (glucose-6-phosphate dehydrogenase, EC 1.1.1.49; A) *Hk* (hexose kinase, EC 2.7.1.1; I) *Lap* (leucine aminopeptidase, EC 3.4.11.1; I); *Ldh* (lactate dehydrogenase, EC 1.1.1.27; D); *Mdh-2* (C); *Pep* (peptidase, phenylalanyl-prolyl substrate; C); *Pgd* (6-phosphogluconate dehydrogenase, EC 1.1.1.44; D).

We resolved allozyme products of 30 presumptive genetic loci from *A. versicolor* queens. Only *Pgm* was polymorphic with 2 alleles designated F (fast migrating) and S (slow). Best resolution was given by buffer D, run for 30 min at 250 V. The monomorphic loci were (enzyme trivial name and EC code [if not listed above] and best buffer are listed after each): *Aat-1* and *Aat-2* (D); *Ac* (D); *Ada* (adenosine deaminase, EC 3.5.4.4; D); *Ak* (Adenylate kinase, EC 2.7.1.20; A); *Ald* (aldolase, EC 4.1.2.13; D); *Ao* (D); *Apk* (arginine phosphokinase, EC 2.7.3.3; D); *Dia* (I); *Est* (D); *Fum* (fumarase, EC 4.2.1.2; D); *Gapdh* (glyceraldehyde phosphate dehydrogenase, EC 1.2.1.12; C); *Gldh* (glucose dehydrogenase, EC 1.1.1.47; A); *Gpi* (C); *G3p* (A); *G6pdh* (B); *Hbdh* (hydroxybutyrate dehydrogenase, EC 1.1.1.30; D); *Hk* (C); *Idh-1* and *Idh-2* (C); *Lap* (C); *Ldh* (B); *Mdh-1* and *Mdh-2* (C); *Me* (malic enzyme, EC 1.1.1.40; A); *Pep* (glycyl-leucyl substrate; C); *Pgd* (A); *Sod* (superoxide dismutase, EC 1.15.1.1; D); *Sordh* (sorbitol dehydrogenase, EC 1.1.1.14; I).

Statistical analyses. The within group relatedness for foundress associations was calculated from the relationship:

$$r = 2*F_{st}/(1 + F_{it}),$$

where F_{st} and F_{it} are Wright's inbreeding coefficients (Hamilton 1972, Pamilo 1984, McCauley et al. 1988). The F_{st} and F_{it} were estimated for each polymorphic locus using Long's (1986) proce-

Table 1. Genotype and allele frequencies of *V. pergandei* queens from foundress associations, separated by subsite.

	EST-1			EST-2			PGM			MDH-1		
Group	CC	CA	AA	FF	FS	SS	FF	FS	SS	FF	FS	SS
Main:												
VF 1	2	2	6	7	3	0	10	0	0	8	2	0
VF 2	1	0	1	1	1	0	2	0	0	2	0	0
VF 3	0	3	1	4	0	0	2	2	0	4	0	0
VF 4	0	3	2	1	3	1	5	0	0	5	0	0
VF 5	0	0	2	2	0	0	2	0	0	2	0	0
VF 6	1	2	1	2	2	0	4	0	0	4	0	0
VF 7	0	3	0	1	2	0	3	0	0	3	0	0
VF 8	1	2	1	2	2	0	4	0	0	4	0	0
VF 9	0	2	3	4	0	1	3	1	0	4	1	0
VF 10	0	3	2	2	2	1	5	0	0	5	0	0
VF 11	0	3	0	2	1	0	3	0	0	3	0	0
VF 12	1	0	1	1	1	0	2	0	0	1	1	0
VF 13	0	2	0	1	1	0	2	0	0	2	0	0
VF 14	0	1	1	0	2	0	2	0	0	2	0	0
VF 15	0	0	2	0	2	0	2	0	0	2	0	0
VF 16	1	1	0	2	0	0	2	0	0	2	0	0
VF 17	-	-	-	3	2	0	5	0	0	4	1	0
VF 18	-	-	-	2	0	0	2	0	0	2	0	0
VF 19	0	2	4	5	0	1	6	0	0	5	1	0
VF 20	2	1	0	2	1	0	1	2	0	3	0	0
VF 21	-	-	-	6	1	0	7	0	0	5	2	0
VF 22	-	-	-	1	1	0	2	0	0	2	0	0
VF 23	-	-	-	2	1	0	2	0	1	3	0	0
	Freq. (A) = 0.66			Freq. (F) = 0.79			Freq. (F) = 0.96			Freq. (F) = 0.95		
Granite:												
VF 24	-	-	-	3	0	0	3	0	0	2	1	0
VF 25	1	3	0	2	1	1	4	0	0	4	0	0
VF 26	0	2	0	1	1	0	2	0	0	1	1	0
VF 27	-	-	-	0	1	1	2	0	0	2	0	0
VF 28	1	1	1	1	2	0	3	0	0	3	0	0
VF 29	1	1	1	2	1	0	3	0	0	2	1	0
VF 30	1	1	0	2	0	0	2	0	0	2	0	0
VF 31	0	4	0	3	1	0	4	0	0	3	1	0
	Freq. (A) = 0.44			Freq. (F) = 0.76			Freq. (F) = 1.00			Freq. (F) = 0.91		

dure, which is corrected for sample size bias. Since all of the polymorphic loci detected in this study had 2 alleles, the method is essentially identical to that of Pamilo (1984, Crozier et al. 1984, Pamilo and Rosengren 1984), when groups are weighted by the number of individuals. Weighting of groups by size appears preferable in the case of *V. pergandei* and *A. versicolor* foundress associations, which varied from 2 to 15 queens in these samples.

Standard errors (S.E.) for the relatedness estimates were obtained by a jacknife procedure over groups (Sokal and Rohlf 1981, Pamilo 1984, Crozier et al. 1984). Simulation studies have shown that S.E. estimated by this method tend to be overly conservative and can be unreliable when allele frequencies are highly unequal (Crozier et al. 1984, Wilkinson McCracken 1985). Because of this, use of these S.E. in formal statistical hypothesis testing is not justified with the present data. In the case of *V. pergandei* foundress associations, a more robust estimate of r is possible by combining estimates across the 4 informative loci (Wilkinson and McCracken 1985). For this pooled estimate we used the weighted means of F_{st} and F_{it} across loci (Long 1986).

Results

Veromessor pergandei. Relatedness within *V. pergandei* foundress associations does not differ from 0 (Table 1; mean estimate across loci = 0.033, Table 2). Allele frequencies of *V. pergandei* are similar between subsites (Table 1); therefore, we treat them as a single population. Pooling subsites would inflate estimated relatedness if subsites differed. *Pgm* and *Mdh-1* allele frequencies are highly unequal, which limits their usefulness as genetic markers for relatedness; they are most informative in combination with *Est-1* and *Est-2* alleles. None of the loci appear linked.

Acromyrmex versicolor. Genetic relatedness among *A. versicolor* co-foundresses is no greater than that expected from randomly associating queens (Table 2, 3). Negative value of the estimate (–0.125) is likely a statistical artifact resulting from unequal *Pgm* allele frequencies and relatively small numbers of queens in each foundress association (mean = 3.8) (Crozier et al. 1984, Wilkinson and McCracken 1985) rather than an indication that queens avoid kin (Hamilton 1972). The small standard error associated with the estimate (0.028) indicates little variation in relatedness among groups, consistent with purely random mixing of genotypes.

Table 2. Relatedness and F-statistics for *V. pergandei* and *A. versicolor* foundress associations. The S.E. is standard error of relatedness, r, based on jacknifing over N groups of foundresses or nestmates. The mean r for *V. pergandei* foundresses is calculated from the weighted mean F_{st} and F_{it} across loci.

	LOCUS	r	S.E.	F_{st}	F_{it}	N
V. pergandei	*Est-1*	0.043	0.106	0.0208	-0.0308	24
	Est-2	-0.009	0.093	-0.0049	0.0527	31
	Mdh-1	-0.117	0.043	-0.0554	-0.0561	31
	Pgm	0.174	0.158	0.1107	0.2685	31
	Mean	0.033		0.0176	0.0638	
A. versicolor	*Pgm*	-0.125	0.028	-0.0632	0.0077	26

Discussion

Foundress associations of *V. pergandei* and *A. versicolor* are not composed of close kin. *Veromessor pergandei* and *A. versicolor* foundresses do not deviate from random assortment of genotypes, precluding the operation of kin selection (Wilson 1977, 1983; Wade 1985). Similar random association of genotypes occurs in polygynous *Solenopsis invicta* colonies (Ross and Fletcher 1985), which are likely founded cooperatively. No other cooperatively founding ant species have demonstrated behavioral evidence of preferential association among relatives (Rissing and Pollock 1988), suggesting that results from the three species now studied electrophoretically are likely to be general. The genetic basis for cooperative behavior among co-founding queens, therefore, cannot be described as a direct consequence of kin selection.

Genetic diversity of many Hymenoptera is lower than found in other insect orders owing either to haplo-diploid sex determination (increasing selective pressure on deleterious alleles exposed in haploid males or decreasing effective population size) or behavioral/environmental peculiarities characteristic of many species, especially social ones (social structure lowering effective population size and providing a nest structure that buffers environmental variability) (reviewed in Graur 1985, Sheppard and Heydon 1986). The decreased variability of *A. versicolor* relative to *V. pergandei* may reflect differences in the mating systems of the two: while all *A. versicolor* colonies in an area release alates on a single day (Wheeler 1917, Rissing et al. 1986), *V. pergandei* colonies release alates over a three month period with little coordination of reproduction among

Table 3. *Pgm* genotype frequencies of *A. versicolor* queens from foundress associations. Groups are listed from largest to smallest in size; the frequencies among 14 solitary queens are given for comparison.

Foundress Group	*Pgm* Genotype		
	FF	FS	SS
FA 4	12	3	0
FA 6	11	2	0
FA 13	9	3	0
FA 5	9	2	0
FA 2	4	0	1
FA 1	3	1	0
FA 3	3	1	0
FA 17	4	0	0
FA 25	3	1	0
FA 34	4	0	0
FA 7	2	1	0
FA 8	3	0	0
FA 10	3	0	0
FA 11	3	0	0
FA 12	3	0	0
FA 14	3	0	0
FA 31	2	1	0
FA 32	2	1	0
FA 33	3	0	0
FA 35	3	0	0
FA 9	2	0	0
FA 15	1	1	0
FA 16	1	1	0
FA 18	2	0	0
FA 19	2	0	0
FA 21	1	1	0
Total:	98	19	1
Solitary:	13	1	0

adjacent colonies (Pollock and Rissing 1985). Under the latter system, small numbers of reproductives (especially males) released per day enhance sampling error associated with the distribution of genes with colonies, thus enhancing genetic variance within a population.

Starting colonies of four North American ant species (*Myrmecocystus mimicus, Solenopsis invicta, V. pergandei* and *A. versicolor*) have clumped starting nests, yet adult colonies of these species are highly territorial, leading to strong intraspecific competition among starting colonies in the form of brood raiding (reviewed in Pollock

and Rissing, 1988b.) Colonies initiated with more foundresses produce more workers and are more likely to succeed at brood raiding (Bartz and Hölldobler 1982, Tschinkel and Howard 1983, Rissing and Pollock 1987). Under such circumstances group life can be considered mandatory, and relatedness of a potential co-foundress is of little or no importance compared to her ability to contribute to an initial brood-raiding force (Pollock and Rissing, 1988a). While relatedness appears to play an important role in the formation of many wasp foundress associations (references above), this appears increasingly unlikely in most ant foundress associations.

Summary

Genetic relatedness among queens within foundress associations of two desert ant species was assayed with protein electrophoresis. Of 18 loci screened in the seed-harvester *Veromessor pergandei,* 4 were variable leading to a within-foundress-association relatedness estimate of $r = .03$ (i.e. random association of queens). Only one locus of 30 screened in the leaf-cutter *Acromyrmex versicolor* was variable. Relatedness within *A. versicolor* foundress associations was estimated at $r = -.12$, with the negative value likely a statistical artifact rather than an indication that kin avoid each other. These data are consistent with behavioral and electrophoretic observations of these and other ants and suggest kin selection plays little, if any, role in formation of most ant foundress associations.

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